(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(4) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section, except prepare a solution containing 3 milligrams of aztreonam per milliliter of methanol and use 0.5 milliliter of the solution as the sample.

[54 FR 40385, Oct. 2, 1989]

§ 455.4a Sterile aztreonam.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam is a practically odorless, white to slightly off-white fine powder. It is sparingly soluble in water of pH 2, and is very soluble at pH values above 4. Its solubility is slight to very slight in polar organic solvents such as methanol and ethanol and it is insoluble in non-polar solvents such as hexane and heptane. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of aztreonam per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 2.0 percent.

(v) Its residue on ignition is not more than 0.1 percent.

(vi) Its heavy metals content is not more than 30 parts per million.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requirements for certification: samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, residue on ignition, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.361 of this chapter, except in lieu of the guard column described in paragraph (a)(4) of that section, use a 5- to 10-centimeter precolumn having an inside diameter of 2 millimeters and packed with octadecyl silane chemically bonded to silica gel of a controlled surface porosity that has been bonded to a solid spherical core (U.S.P. designation L–2) 30 micrograms to 50 micrograms in diameter; and use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating at a wavelength of 270 nanometers (or 254 nanometers fixed mercury source), a column packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles (U.S.P. designation L–1) 5 micrograms to 10 micrograms in diameter or equivalent, a flow rate of 1.5 milliliters per minute, and a known injection volume of 20 microliters. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.05M potassium phosphate buffer, pH 3.0.* Prepare a solution containing 6.8 grams of potassium phosphate monobasic per liter of distilled water. Adjust the solution to pH 3.0 with 1*M* phosphoric acid.

(*b*) *Mobile phase.* 0.05*M* potassium phosphate buffer, pH 3.0: methanol (4:1).

(ii) *Preparation of working standard, sample, and resolution test solutions*—(*a*) *Working standard solution.* Transfer approximately 25 milligrams of aztreonam working standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase.

(*b*) *Sample solution.* Transfer approximately 25 milligrams of the sample, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase.

(*c*) *Resolution test solution.* Dissolve 10 milligrams of [2*S*-[2*alpha*,3*beta*(*E*]]-2-

[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene] amino]oxy]-2-methylpropanoic acid (E isomer) in 10 milliliters of working standard solution and dilute to 50-milliliters with mobile phase.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 2 at 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates.

(*c*) *Resolution.* The resolution (*R*) between the peak for aztreonam and the E isomer is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.361(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculation.* Calculate the micrograms of aztreonam per milligram as follows:

$$\text{Micrograms of aztreonam per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the aztreonam peak in the chromatogram of the aztreonam working standard;

P_s=Aztreonam activity in the aztreonam working standard solution in micrograms per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid I in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of aztreonam and 39 milligrams of pyrogen-free L-arginine base per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section, except prepare a solution containing 3 milligrams of aztreonam per milliliter of methanol and use 0.5 milliliter of the solution as the sample.

[52 FR 4614, Feb. 13, 1987, as amended at 55 FR 11584, Mar. 29, 1990]

§ 455.10 Chloramphenicol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol is a white to grayish-white or yellowish-white powder, occurring as needles or elongated plates. It is neutral, slightly soluble in water, but freely soluble in alcohol. It has the chemical formula D-(—)-*threo*—1-*p*—nitrophenyl-2 - dichloracetamido-1,3-propanediol. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its pH in a saturated aqueous solution is not less than 4.5 nor more than 7.5.

(iv) Its specific rotation in absolute ethyl alcohol at 20° C. is +20°±1.5°, and at 25° C. is +18.5°±1.5°.

(v) Its melting range is 151°±2° C.

(vi) Its absorptivity at 278 nanometers is 100 ±3 percent of that of the chloramphenicol working standard similarly treated.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain: